Supporting Information

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SI Materials and Methods

Reagents and Antibodies. Streptavidin-HRP was from Pierce. Histone H2B antibody was from Upstate Biotechnology. FLAG, actin, and tubulin antibodies were obtained from Sigma. FLAG-agarose also was from Sigma. Antibodies to GFP and TATA box binding protein (TBP) associated factor 1 (TAF1) were from Santa Cruz Biotechnology. Antibody to acetylated lysine (Lys) was from Cell Signaling. Antibody to heat shock factor 1 (HSF1) was from Stressgen.

Plasmids. The plasmids for recombinant histone 3 (H3) and histone 4 (H4) production were a kind gift from Hitoshi Kurumizaka (RIKEN Genomic Sciences Center, Yokohama, Japan). H3 and H4 were expressed, purified, and renatured as previously described (1). The pME18S-FLAG histone constructs were a generous gift from Robert N. Eisenman (Fred Hutchinson Cancer Research Center, Seattle). The plasmid for OGT expression in *Escherichia coli* was a kind gift from Suzanne Walker (Harvard Medical School, Boston). Site-directed mutagenesis was performed using the Stratagene Quikchange II XL kit according to manufacturer's instructions.

Cell Culture. HeLa cells were maintained in DMEM supplemented with 10% FBS and penicillin/streptomycin in a humidified atmosphere at 37 °C. To obtain hyperacetylated histones, HeLa cells were treated overnight with 10 mM sodium butyrate (Sigma). For mitotic cells, HeLa cells were treated overnight in 80 ng/mL nocodazole (Sigma). Mitotic cells were collected by mitotic shake-off. To obtain a late mitotic extract, cells obtained by mitotic shake-off were washed twice with PBS and replated onto poly-D-lysine–coated plates for 1 h at 37 °C. Early G1 cells were harvested 3 h after replating. Double thymidine block was performed as described (2). Cells were transfected using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). Control siRNA and siRNA directed to TAF1 was obtained from Dharmacon. HeLa cells were infected with adenovirus to GFP or to β-*N*-acetylglucosamine (O-GlcNAc) transferase (OGT) at a multiplicity of infection of 100. For heat-shock experiments, HeLa cells were plated at 1×10^6 cells per 10-cm dish. Cells were allowed to recover for 1 d. The following day, the medium was changed 1 h before heat shock. Cells were stressed for 1 h at 45 °C and then allowed to recover at 37 °C. Control cells were harvested at the same time as the 0-min recovery samples. For heat shock experiments with adenoviral infection, cells were infected the day after plating.

Acid Extraction of Histones. Histones were acid extracted by resuspending cells in lysis buffer containing 10 mM Tris (pH 7.5), 2 mM $MgCl_2$, 3 mM $CaCl_2$, 1% octyl phenoxylpolyethoxylethanol (Nonidet P-40) supplemented with inhibitors (Protease Inhibitor Cocktail 1 [PIC1], 10 mM sodium butyrate, 1 mM NaF, 1 mM β-glycerophosphate, and 2 μM O-[2-Acetamido-2-deoxy-D-glucopyranosylidene]amino N-phenyl carbamate [PUGNAc]). Nuclei were obtained by using a Dounce homogenizer and centrifugation. Nuclei were washed twice in lysis buffer, once in lysis buffer containing 100 mM NaCl, once in lysis buffer containing 400 mM NaCl, and once in 10 mM Tris (pH 7.5), 2 mM $MgCl_2$, and 3 mM $CaCl_2$. All buffers contained inhibitors. Then the cell pellet was resuspended in water, and sulfuric acid was added to obtain a final concentration of 0.2 M. After incubation on ice for ≈1 h, the lysate was clarified by centrifugation. The supernatant containing histones was dialyzed into 0.1 *N*-acetic acid two times for 2 h before being dialyzed into water for 1 h, 3 h, and overnight. After centrifugation to remove any precipitated material, the histones were lyophilized.

Purification of Native Histones. Native histones were purified by resuspending the cell pellet in Hepes buffer (HB) [20 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM β-mercaptoethanol, 10% glycerol, and inhibitors] containing 0.4 M NaCl. After incubation on ice, nuclei were pelleted at $20{,}000 \times g$ for 5 min. This step was repeated an additional four times. The pellet then was washed two times in HB containing 0.4 M NaCl and 0.2% Nonidet P-40 and two times in hydroxylapatite buffer (HAP) [50 mM sodium phosphate (pH 6.8), 1 mM β-mercaptoethanol, and inhibitors] containing 0.4 M NaCl. Finally, before sonication the nuclei were resuspended in HAP containing 0.6 M NaCl. Native histones then were purified over a hydroxylapatite column and washed extensively with HAP containing 0.6 M NaCl. Histones were eluted with HAP containing 2.5 M NaCl. The eluate was dialyzed into 20 mM Tris (pH 8.0), 0.25 mM EDTA, and 2 M NaCl. Histones were more than 95% pure based on Coomassie G-250 staining.

Galactosyltransferase Assays. *N*-acetylglucosamine (GlcNAc) moieties were detected using the Click-IT kit (Invitrogen) according to the manufacturer's instructions. For detection using ^{3}H-Gal, histones were incubated with galactosyltransferase (GalT) (Sigma) and 1 μCi UDP-[^{3}H]-Gal in 10 mM Hepes (pH 7.5), and 5 mM $MnCl_2$ overnight at 4 °C. Reactions were terminated with Laemmli buffer and separated by SDS/PAGE. After fixing and staining with Coomassie G-250, gels were treated with En^{3}Hance autofluorography reagent (Perkin-Elmer), dried, and exposed to film.

Recombinant OGT Assays. Recombinant OGT was expressed and purified as previously described (3). OGT assays were performed in 50 mM Tris (pH 8.0) and 1 μCi UDP-[^{3}H]-GlcNAc. Reactions were terminated with Laemmli buffer and separated by SDS/PAGE. Gels were fixed, stained with Coomassie G-250, treated with En^{3}Hance autofluorography reagent, dried, and exposed to film.

In-Gel Digest, Chemoenzymatic Tagging, and Chemical Derivatization. Histones were resolved by SDS/PAGE and stained by Commassie G-250. Histone bands were excised and digested following a standard in-gel digestion protocol (4) without reduction and alkylation. Tryptic peptides were treated with 50 units of calf intestinal phosphatase (New England Biolabs) for 4 h at 37 °C. UDP-azide-modified galactosamine (UDP-GalNAz) (Invitrogen) was added (~2× in excess) and incubated overnight with mutant GalT1 and 2,000 unit/mL peptide *N*-glycosidase F (PNGase F) (New England Biolabs) in 50 mM NH_4HCO_3. After the reaction, excess UDP-GalNAz was removed by passing the mixture through a C18 spin column (Nest Group). Peptides were eluted in 80% acetonitrile. Cycloaddition reaction was performed in a volume of 20 μL containing biotin-PEG-alkyne (~3× in excess, dissolved in DMSO; Invitrogen), 2 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 2 mM Tris [(1-benzyl-^{11}H,2,3-triazol-4-yl) methyl] amine (TBTA), and 2 mM $CuSO_4$. The reaction mixture was incubated for 12 h at room temperature with gentle shaking. After the reaction, the solution was allowed to bind to avidin-agarose beads (Pierce) for 2 h at room temperature, followed by extensive washing. β-elimination and Michael addition were performed directly on the bead using the protocol previously described (5). Peptides in the supernatant were cleaned up by C18 spin column (Nest Group).

Mass Spectrometry and Data Analysis. An LTQ (Thermo Finnigan) ion trap mass spectrometer coupled with a nano-2D liquid

chromatography (LC) pump (Eksigent Technologies) was used to analyze the derivatized peptides. Peptides were eluted from a C18 column with a 40-min gradient and sprayed into the spectrometer. The spectrometer was programmed to record a full precursor scan (350–1,800 m/z) followed by fragmentation and MS/MS scans of the eight most intense ions. The raw data were searched against the SwissProt database using Mascot (version 2.1.0). The following parameters were used: Homo sapiens as species, DTT (ST) and deamination (NQ) as variable modification, no fixed modification. Precursor and fragment ion mass tolerances were 1.2 atomic mass units and 0.8 atomic mass units, respectively.

Western Blotting and Immunoprecipitation. For immunoprecipitation of FLAG-tagged histones, cells were resuspended in 20 mM Tris (pH 8.0), 600 mM NaCl, 0.5% Nonidet P-40, 0.5% SDS, 0.5% deoxycholate, and 1 mM EDTA supplemented with inhibitors. The cells then were sonicated, and the lysate was clarified by centrifugation. The lysate was diluted with 10 mM Tris (pH 8.0), 150 mM NaCl, and 1% Triton X-100 supplemented with inhibitors, and histones were immunoprecipitated with FLAG-agarose. Immunoprecipitates were washed three times with lysis buffer before complexes were eluted with Laemmli buffer. For small-scale purification of histones, soluble nucleocytoplasmic proteins were extracted by resuspending cells in 10 mM Tris (pH 8.0), 150 mM NaCl, and 1% Nonidet P-40 supplemented with inhibitors. Insoluble material was pelleted by centrifugation. The insoluble pellet was washed with hypotonic lysis buffer [10 mM Hepes (pH 7.9), 10 mM KCl, and 1.5 mM $MgCl_2$ supplemented with inhibitors] before acid extraction with HCl.

2D Gel Analysis. Acetic acid urea (AU) gel electrophoresis was performed as described (6). Briefly, acid-extracted histones were precipitated with chloroform and methanol and resuspended in AU sample buffer (72% urea, 0.04% Pyronin Y, 10% acetic acid, and 12.5 mg protamine sulfate). Samples were electrophoresed on AU gels (36% urea, 15%:0.1% acrylamide:bisacrylamide, 5% acetic acid) that had been prerun overnight with AU sample buffer. Lanes were excised and then equilibrated in 125 mM Tris (pH 8.8) before separation by SDS-PAGE.

***In Nucleo* OGT Assays and OGT Peptide Assays.** Nuclei were prepared as described (7). Briefly, cells were incubated in 20 mM Tris (pH 8.0), 3 mM $MgCl_2$, 10 mM NaCl, and 0.05% Nonidet P-40 for 5 min on ice. Nuclei were pelleted by centrifuging for 5 min at $600 \times g$. For *in nucleo* OGT assays, nuclei were resuspended with 20 mM Tris (pH 8.0) and 1 μCi ^{3}H-UDP-GlcNAc and were incubated for 1 h at room temperature. Reactions were terminated with Laemmli buffer and separated by SDS/PAGE. Gels were fixed, stained with Coomassie G-250, treated with En^{3}Hance autofluorography reagent, dried, and exposed to film. For OGT assays against peptide substrate, isolated nuclei were lysed in 10 mM Tris (pH 8.0), 150 mM NaCl, and 1% Nonidet P-40 supplemented with inhibitors. Lysates were clarified by centrifugation, and OGT was immunoprecipitated for 2 h at 4 °C with constant rotation. Protein G beads (GE Healthcare) were added, and immunoprecipitation continued for 1 h at 4 °C. Beads were washed three times with lysis buffer and twice with 20 mM Tris. Reactions were performed in triplicate as described in ref. 8.

MNase Chromatin-Accessibility Assays. Cells were resuspended in buffer A [20 mM Tris (pH 7.5), 1.5 mM $MgCl_2$, 340 mM sucrose, and 10% glycerol] at $\approx 2 \times 10^7$ cells/mL An equal volume of buffer A with 0.4% Triton X-100 was added, and cells were incubated on ice for 5 min. Nuclei were collected by centrifugation at $600 \times g$ for 5 min at 4 °C. Nuclei were washed once with buffer A before being resuspended in buffer A with 2 mM $CaCl_2$. Nuclei were digested with MNase (New England Biolabs) for 0, 2, 5, 10, and 15 min (2 μg DNA per time point). Reactions were terminated by adding 50 mM Tris (pH 8.0), 1% SDS, 40 mM EDTA, and 200 mM NaCl. Reactions were digested with RNase A and Proteinase K before DNA was extracted with phenol/chloroform. DNA was precipitated before resolving on a 1.2% agarose gel.

Immunofluorescence. HeLa cells transfected with histone constructs were fixed in 4% paraformaldehyde for 20 min at room temperature. Cells then were permeabilized in 0.5% Triton X-100 in for 10 min at room temperature before being washed twice in wash buffer (0.1% Triton X-100 in PBS). Cells were blocked for 1 h at room temperature in antibody dilution buffer (2% BSA, 0.1% Triton X-100 in PBS). Primary antibody incubations overnight or for 2 h at room temperature were done sequentially. Cells were washed three times before incubation in secondary antibody (Alexa Fluor 647 and Alexa Fluor 488; Invitrogen) for 1 h at room temperature. The nucleic acid stain LOLO-1 (Invitrogen) was included in the secondary antibody incubation. Cells were washed five times in wash buffer and twice in PBS before being mounted on coverslips (Vectashield; Vector Laboratories). Images were obtained on a 3I Spinning Disk Confocal microscope using Olympus Slidebook software at the Johns Hopkins University School of Medicine Core Microscopy Facility.

Statistical Analysis. All experiments were performed on at least three separate occasions ($n \geq 3$). In all figures, error bars represent SE. P values were calculated using the paired two-tailed Student's t test.

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Fig. S1. Histones are in vitro substrates for OGT. (*A*) Acid-extracted histones were subjected to an in vitro OGT assay. Upper gel shows the total protein load. Lower gel is the autoradiograph. Arrow indicates auto-O-GlcNAyclated OGT. (*B*) Purified native histones also are a substrate for OGT. (*C*) Renatured recombinant H3 and H4 also are substrates for OGT.

Fig. S2. Schematic for identifying O-GlcNAc sites on histones. BEMAD, β-elimination followed by Michael addition with DTT. S/T, Ser/Thr.

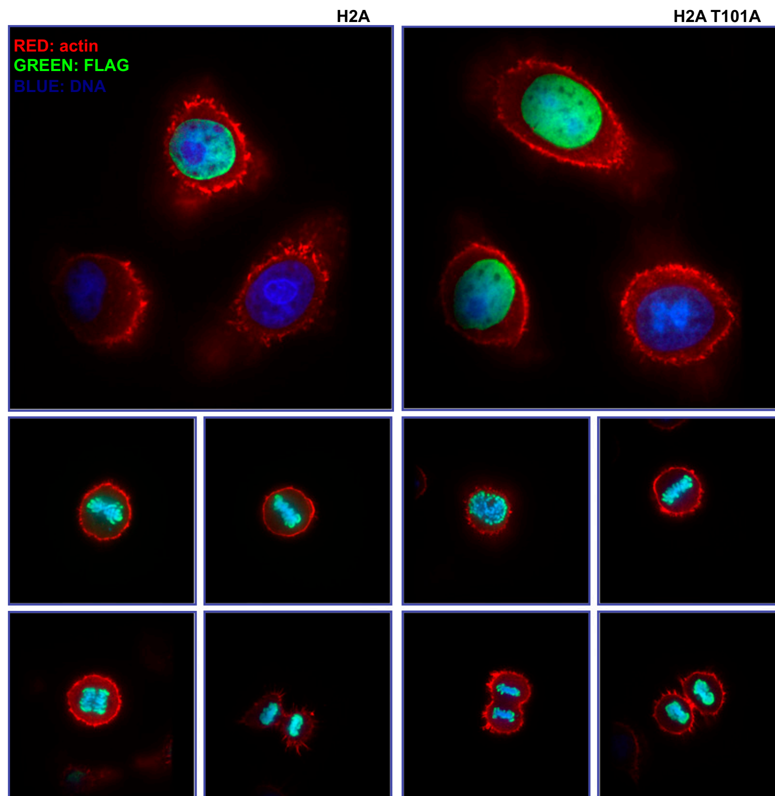

Fig. S3. FLAG-tagged histones and mutants costain with DNA. HeLa cells were transfected with FLAG-tagged constructs and stained for FLAG (green), actin (red), and DNA (blue). Pictures are representative for all wild type histones and serine/threonine-to-alanine mutants.

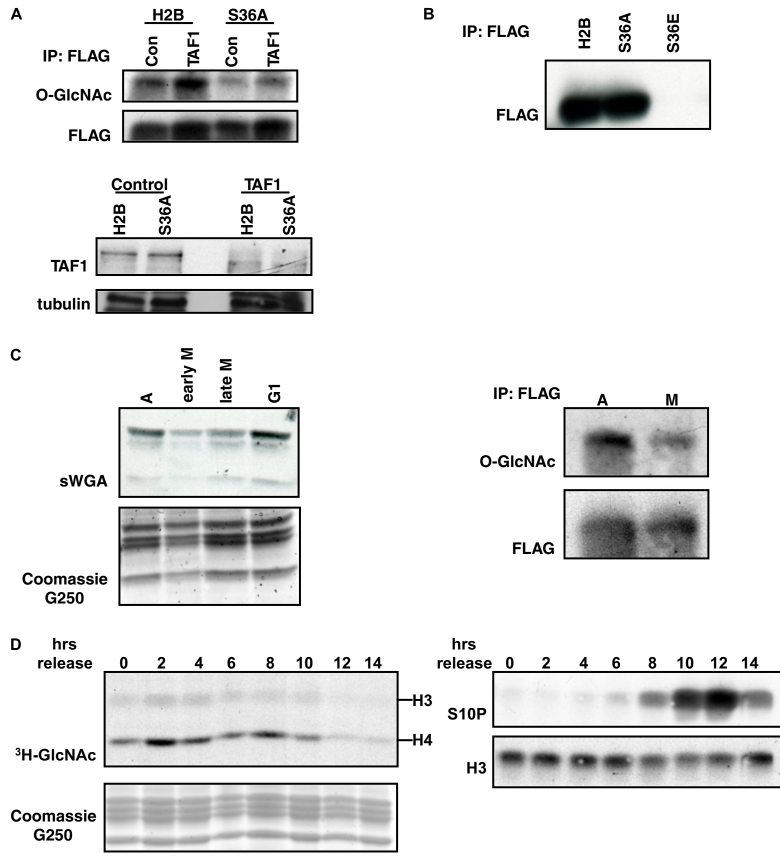

Fig. S4. Histone O-GlcNAcylation and cell cycle. (*A*) Knockdown of TAF1 does not increase O-GlcNAcylation of H2B. S36A, serine 36A. IP, immunoprecipitation. (*B*) Mutation of S36A of H2B to glutamic acid decreases its expression. (*C*) Histone O-GlcNAcylation decreases during the M phase, with H3 being the most affected. sWGA, succinylated wheat germ agglutinin. (*D*) OGT activity toward histones decreases during the M phase as assessed by *in nucleo* OGT assay. Western blot of phosphorylated serine-10 (S10P) of H3 is included to indicate mitotic samples.